NTP
National Toxicology Program

Alkylaniline Class Study

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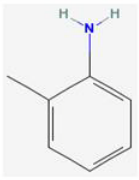
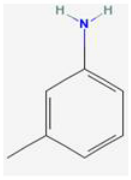
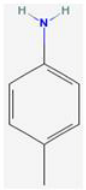
NTP Board of Scientific Counselors
December 9-10, 2009

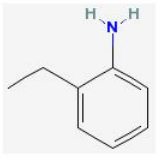
Monomethyl substituted

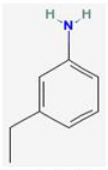

2-methylaniline


3-methylaniline


4-methylaniline

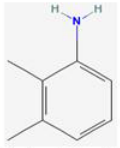
Monoethyl substituted

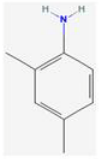

2-ethylaniline

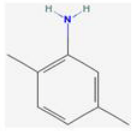

3-ethylaniline

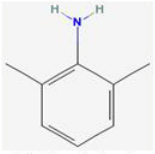

4-ethylaniline

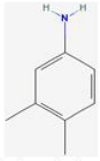
Dimethyl substituted

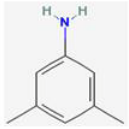

2,3-dimethylaniline


2,4-dimethylaniline

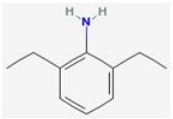

2,5-dimethylaniline

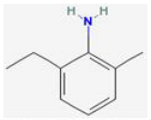

2,6-dimethylaniline


3,4-dimethylaniline


3,5-dimethylaniline

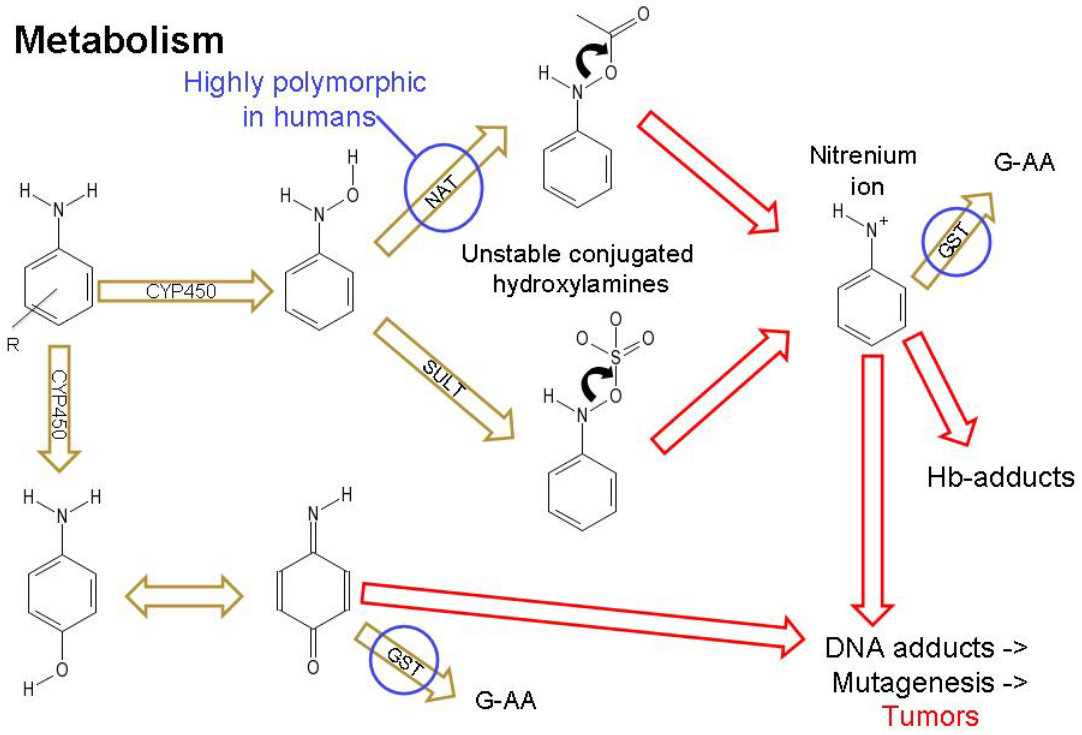
Others with HPV status


2,6-diethylaniline


2-methyl-6-ethylaniline

Alkylanilines - Background

- Ubiquitous potential exposure scenarios
  - Combustion products
- Exposure has been documented in humans based on the hemoglobin (Hb) adducts
- Hb adduct levels have been associated with bladder cancer in humans
- Carcinogenic in rodents (Liver, Bladder, Subcutaneous tissue, Nasal Cavity)
- Form DNA adducts in vivo (Liver, Bladder and Nasal Cavity)
- DNA adduct formation is a byproduct of metabolic activation


Metabolism
Highly polymorphic in humans
NAT
CYP450
CYP450
SULT
Unstable conjugated hydroxylamines
Nitrenium ion
G-AA
GST
Hb-adducts
GST
G-AA
DNA adducts ->
Mutagenesis ->
Tumors

Proposed Studies

- *In vitro* and *In vivo* DNA adduct and mutagenesis studies
  - Compare genotoxic potency
- Evaluate select subset of alkylanilines in short-term carcinogenicity bioassay
  - Determine relationship between genotoxic and carcinogenic potency
- **Determine contribution of genetic variability to genotoxic potency for those alkylanilines exhibiting high levels of genotoxicity**

Proposed Approach

- Perform genotoxicity studies using cultured hepatocytes from multiple inbred strains of mice
  - Comet Assay
    - Quantify DNA Damage
  - DNA adduct formation
    - Quantify total radioactivity associated with DNA
  - Use genotoxicity endpoints to identify QTL (in silico mapping) that determine differential susceptibility to genotoxic effects of alkylanilines

Key Issues

- Why hepatocytes?
  - Alkylanilines are carcinogenic in rodent liver
  - Alkylanilines form DNA adducts in mouse liver
  - Mechanism of carcinogenesis in liver is plausibly related to carcinogenic effect in bladder
    - Formation of unstable conjugated hydroxylamines or quinoneimines
- Which strains of mice?
  - **Initial study:** Selection will be based on genetic diversity at loci coding for proteins involved in arylamine metabolism
    - *Cyp1a2*, *Nat1*, *Nat2* and *Gstm1*
  - If the phenotype exhibits significant variation in initial study:
    - Evaluate 30 to 50 strains and perform HAM

Expected Outcomes

- Quantify the degree to which genetic variation can impact alkylaniline-associated genotoxicity
  - Provide better estimates of interindividual risk
  - Help identify susceptible subpopulations which can be applied to alkylaniline hazard characterization

Current Activities

- Evaluate 14 alkylanilines for genotoxic potency using an AS52 cell assay
  - Hypoxanthine-gaunine phosphoribosyl transferase (HPRT) deficient Chinese Hamster Ovary (CHO) cells that have stably integrated bacterial xanthine-guanine phosphoribosyl transferase (gpt) gene
  - Mutations in gpt confer resistance to 6-thioguanine
  - Can be done in the presence S9 fraction